In the Office Action mailed in the present case on February 13, 2001, claims 1-3 and 12-31 were examined. Claims 1-3 and 21-31 were rejected under 35 U.S.C. § 112, first paragraph, and 35 U.S.C. § 112, second paragraph. Claims 1, 3, and 31 were also rejected under 35 U.S.C. § 102(b). In the Advisory Action, mailed August 23, 2001, the Examiner stated that the amendments, filed August 13, 2001, were not entered. The rejections raised in the Office Action are addressed below.

Support for the Amendments

Subject matter from canceled claim 1 has been presented in new claim 32 to more clearly recite the invention the Applicants intend to claim. Support for the amendments to claims 3 and 12-31 is found throughout the specification, for example, at page 16, lines 15-25, and at page 23, line 23 through page 25, line 5. Support for new claims 32 and 33 is found in the specification at page 8, lines 11-13.

In the interest of expediting prosecution, claims 1 and 2 have been canceled, and claims 3 and 12-31 have been amended to remove reference to subfragments or optimized fragments. For the record, applicants do not agree with the present rejection of claims 1-3 and 12-31 and reserve the right to pursue the canceled subject matter in this or a related continuing application. Applicants also note that the removal of references to subfragemnts and optimized fragments in the claims does not require a new search by the Examiner because no additional subject matter is being claimed.

No new matter is added by the amendments.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-3 and 12-31 were rejected under 35 U.S.C. § 112, first paragraph, for failure of the specification to convey possession of the scope of the claimed invention. The Examiner states that the specification does not disclose which RNA binding proteins are bound by the nucleic acid sequences of SEQ ID NOS: 1-20 or whether any of the sequences affect the functional characteristics of the mRNA with which it is associated. The Examiner further states that the specification does not disclose where the nucleic acid sequences of SEQ ID NOS: 1-20 are located within the context of the genes from which they were obtained. The Examiner also argues that the specification does not provide enough description of the structural/functional characteristics to allow one of skill in the art to extrapolate that data to a representative number of embodiments for the genus in which the nucleic acid sequences of SEQ ID NOS: 1-20 are linked to heterologous sequences. Applicants respectfully disagree.

Rejection for failure to describe RNA binding proteins or disclose regulatory function.

As stated on page 11, lines 7-26, of the specification, a standard assay was used to demonstrate the ability of the nucleic acid sequences of SEQ ID NOS: 1-20 to bind RNA binding proteins (RBPs). Numerous examples of other standard methods that may be

used to detect RNA/RBP interactions are provided in the specification. Any of these assays may be used by one skilled in the art to determine which RBPs are bound by each of the nucleic acid sequences of SEQ ID NOS: 1-20. For example, as stated on page 13, lines 6-20, of the specification:

> The methods of detecting such RNA/RBP binding pair interactions are well known in the art, and include, for example, filter binding assays (Wu and Uhlenbeck, Biochemistry 26: 8221-8227, 1987; Carey and Uhlenbeck, Biochemistry 22: 2610-2615, 1983), electrophoretic gel mobility shift assays (Izquierdo and Cuezva, Mol. Cell. Biol. 17:5255-5268, 1997; Malter, Science 246: 664-666, 1989; Zaidi and Malter, J. Biol. Chem. 269: 24007-24013, 1994; Claffey et al., Mol. Biol. Cell 9: 469-481, 1998; Brewer, Mol. Cell. Biol. 11: 2460-2466, 1991); homopolymer beads (Siomi et al., Cell 77: 33-39, 1994), or fluorescence anisotrophy (Tetin et al., Biochemistry 32: 9011-9017, 1993; Goss et al., Nucleic Acids Research 11: 5589-5602, 1983; and Liang et al., WO 98/39484).
>
> It is preferred that conditions allowing detection of interactions between nearly every type of RNA and RBP pair be employed. Exemplary protocols, binding conditions, and RNA binding proteins that may be used are disclosed in detail in PCT application WO 98/04923 and are summarized below.

The specification also provides several standard assays that may be used to determine the effect of the nucleic acid sequences of SEQ ID NOS: 1-20 on the mRNA molecules with which it is associated (see, for example, pages 16-19). As numerous methods for assaying the RBP binding activity or regulatory function of the nucleic acid sequences of SEQ ID NOS: 1-20 are described and enabled in the specification, this aspect of the rejection may be withdrawn.

Rejection for failure to describe source of UTR sequences.

In response to the Examiner's argument that the specification does not disclose where the nucleic acid sequences of SEQ ID NOS: 1-20 are located within the context of the genes from which they were obtained, Applicants note that the nucleic acid sequences of SEQ ID NOS: 1-20 are 5'UTRs and 3' UTRs from sequences deposited in publically available databases. Based on the disclosed accession numbers for these deposited sequences, one skilled in the art can readily identify the 5'UTRs and 3' UTRs from these deposited sequences. The nucleic acid sequences of SEQ ID NOS: 1-20 are also identified by their exact polynucleotide sequence in the Sequence Listing filed with the application. In view of these clarifying remarks, this aspect of the rejection may now be withdrawn.

Rejection for failure to describe heterologous sequences that allow UTRs to retain RBP binding activity.

As stated on page 16, lines 15-25, of the specification, the nucleic acid sequences of SEQ ID NOS: 1-20 may be operably linked to any heterologous sequence:

> constructs are generated in which the parent or subfragment UTR sequence to be tested is adjoined to the coding segment of a gene at a position upstream or downstream of the coding region, depending on whether the UTR is derived from a 5' or 3' UTR, respectively. The coding region may encode a reporter gene, or the gene that is endogenously associated with the UTR sequence with an inserted tag, such as an epitope tag. Intracellular expression of the construct can be achieved by transfecting cells with

expression vectors containing the construct. If the parent or subfragment sequence is adjoined to a reporter gene, then changes in the level of reporter gene product can be used as an indication that the parent or subfragment sequence regulates at least one aspect of mRNA functionality.

Additionally, the specification states that:

> An additional use for the parent UTR sequences or their optimized subfragments is their incorporation into a recombinant construct such that expression of the construct is controlled by the UTR sequence. For example, a nucleic acid sequence of the invention can be inserted into a heterologous gene to form all or a part of the untranslated region of the gene's mRNA transcript. It is expected that the UTR sequence will interact with an RBP in these recombinant RNA molecules and that protein expression of the heterologous gene will be affected. This is analogous to recombining promoters with heterologous coding regions to alter or control the expression of the coding region.

Page 23, line 23, through page 24, line 5.

Additionally, the signed Declaration of Anthiony Giordano, Ph.D., filed January 28, 2002, provides numerous examples demonstrating the reliability with which a UTR from a gene of interest maintains its regulatory function when operably linked to a heterologous sequence, such as a reporter gene. The examples indicate that the nucleic acid sequences of SEQ ID NOS: 1-20 retain their RBP binding activity and regulatory activity when operably linked to heterologous sequences. For example, Blau et al. (WO 98/42854, published October 1998) states that "[t]he major approach to the study of UTRs to date has been to introduce a UTR-reporter gene construct" into a cell line (page 3, lines 16 and 17). Blau also notes that a prior study demonstrated that the expression of a chimeric gene containing "the 5' regulatory region of β-actin linked to the 3'UTR of α-

skeletal actin does not decline during differentiation, but increases like the endogenous α-skeletal actin gene" (page 3, lines 4-7, a copy of this reference is enclosed). Thus, the 3'UTR of α-skeletal actin maintained its regulatory role when operably linked to a heterologous sequence.

As discussed by Dr. Giordano, Blau also reported the ability of several 3'UTR fragments to maintain their regulatory function when operably linked to a heterologous bacterial β-glucuronidase reporter gene. Blau further notes that using the same reporter gene to study the function of several UTRs allows for a more direct comparison between the UTRs:

> The following example examines the regulatory properties of highly conserved regions (HCRs) within 3'UTRs that have retained greater than 70% homology within stretches of 100 nucleotides over 30 million years. A retroviral vector system is used within a selectable marker that allows rapid delivery of 3'UTR-reporter constructs to populations of thousands of cells within one to two weeks, avoiding problems associated with clonal analysis and long-term selection. Moreover, this vector is modular, thereby permitting direct comparison of different HCRs on gene expression, independent of 5'UTRs, promoters, protein coding regions, and polyadenylation signals. Since the 5'UTR, promoter, coding region and polyadenylation signal are constant in this vector, the influence of HCRs on gene expression can be directly compared. Ten HCRs (c-fos, c-myc, transferrin receptor, bcl2, EF1α, vimentin, ornithine decarboxylase, fibronectin, HuD, and Ran), all of which are associated with proteins with a role in growth control, were examined. Using this methods and compositions of the invention, it is shown that HCRs can cause marked changes in mRNA and protein accumulation under steady state conditions and in response to changes in the cell milieu typical of sites where tumors develop. Nine of ten HCRs were found to decrease mRNA stability, to different extents. Two HCRs altered mRNA translation under steady state conditions. Four HCRs mediated responses to changes in mitogen level by increasing reporter protein levels 2-fold; whereas two HCRs exhibited a 6-

fold difference in their response to another environmental stress, hypoxia. Page 27, line 25, through page 28, line 11.

The Giordano Declaration also provides data demonstrating the ability of the 5'UTR and 3'UTR of Her2 to maintain their regulatory activity when operably linked to the luciferase heterologous sequence. As discussed by Dr. Giordano, in paragraph three of the Declaration, polysome distribution analysis was used to compare the translational efficiency of an endogenous Her2 mRNA gene to the translational efficiency of a chimeric mRNA molecule containing both the 5'UTR and 3'UTR of Her2 operably linked to a luciferase reporter gene (U.S.S.N. 60/278,902; filed March 26, 2001). A chimeric Her2 UTR/reporter construct encoding this chimeric mRNA was stably transfected into the MCF7 breast cancer cell line. The resulting polysome distribution of the chimeric mRNA was similar to that of endogenous Her2 (Figures 1B and 1D containing this data from U.S.S.N. 60/278,902 are attached).

The Giordano Declaration further demonstrates that the 5'UTR and 3'UTR of Her2 modulate the half-life of the chimeric mRNA molecule (U.S.S.N. 60/278,902). As paragraph three of the Declaration states, MCF7 cells stably transfected with a control luciferase construct that did not contain any HER2 UTRs, the half-life of the luciferase mRNA was approximately 4 hours. In contrast, the mRNA corresponding to the stably transfected luciferase construct containing both 5' and 3' HER2 UTRs had a half-life of only approximately 1 hour. These data demonstrate that UTRs operably linked to

heterologous coding sequences can modulate mRNA stability.

As noted in the Giordano Declaration, heterologous sequences, such as reporter genes, can be readily identified by one skilled in the art. Examples of commonly used reporter genes include chloramphenicol acetyltransferase, firefly luciferase, renilla luciferase, β-galactosidase, secreted alkaline phosphatase, human growth hormone, β-glucuronidase, green fluorescent peptide, and red fluorescent protein, among others (see, for example, Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, 1995).

The present invention clearly sets forth a system for operably linking any one of the nucleic acid sequences of SEQ ID NOS: 1-20 to a heterologous sequence and assaying the RBP binding activity and mRNA functionally of the resulting fusion mRNA. It has been established that the specification need not explicitly teach every possible embodiment of the invention. As stated in *Scripps Clinic & Research Foundation v. Genetech, Inc.*, "the purpose of [the enablement] provision is to assure that the inventor provides sufficient information about the claimed invention that a person of skill in the field of the invention can make and use it without undue experimentation, relying on the patent specification and the knowledge in the art," See, 18 USPQ2d 1896, 1006 (Fed. Cir. 1991). Similarly, *In re Vaeck*, states that "[t]he first paragraph of 35 US.C. § 112 requires, inter alia, that the specification of a patent enable any person skilled in the art to which it pertains to make and use the claimed invention. Although

the statute does not say so, enablement requires that the specification teach those in the art to make and use the invention without 'undue experimentation' . . . That some experimentation may be required is not fatal; the issue is whether the amount of experimentation required is 'undue,'" See, USPQ2d 1438, 1444 (Fed. Cir. 1991).

In summary, the specification provides sufficient guidance and working examples so that only reasonable experimentation is required to extend the particular findings to any heterologous sequence operably linked to one of the nucleic acid sequences of SEQ ID NOS: 1-20. The key aspect of the present invention is the discovery that the nucleic acid sequences of SEQ ID NOS: 1-20 have RBP binding activity. Given this information, any skilled artisan would recognize that this finding can be extended to fusion mRNAs in which a heterologous sequence as added to nucleic acid sequences of SEQ ID NOS: 1-20. In light of these teachings, Applicants submit that the specification conveys possession of the claimed invention in its present scope.

Rejection for failure to describe subfragments and optimized sequences.

The Examiner also argues that the specification did not provide sufficient guidance for subfragments or optimized fragments of the nucleic acid sequences of SEQ ID NOS: 1-20. In the interest of expediting prosecution, claims 1 and 2 have been canceled, and claims 3 and 12-31 have been amended to remove reference to subfragments or optimized fragments.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-3 are rejected under 35 U.S.C. § 112, second paragraph, on the ground that they are indefinite for failing to point out and distinctly claim the subject matter that Applicants regard as the invention. The Examiner states that it is unclear whether claim 1 is directed to only the first nucleic acid (one of SEQ ID NOS: 1-20) or the larger nucleic acid of which it is part. The Examiner also states that it is unclear whether the first nucleic acid (one of SEQ ID NOS: 1-20) regulates the functionally of the larger nucleic acid of which it is part. In response to these rejections, Applications have canceled claim 1 and added new claim 32, which recites a fusion nucleic acid that includes a first nucleic acid operably linked to a heterologous second nucleic acid. The mRNA form of this fusion protein may be regulated by the mRNA form of the first nucleic acid. Claims 3 and 12-31 have also been amended to recite a fusion nucleic acid. Accordingly, this rejection may be withdrawn.

Rejection under 35 U.S.C. § 102(b)

Claims 1, 3, and 31 were rejected under 35 U.S.C. § 102(b) based on the assertion that the polynucleotide sequence "tag" present in the polynucleotide sequence of a UTR disclosed by Claffey et al (Mol. Biol. Cell. 9:469-481, 1998) anticipates claims encompassing a subfragment of SEQ ID NO: 20. As noted above, in the interest of

expediting prosecution, the claims have been amended to no longer recite subfragments of SEQ ID NOS: 1-20. Accordingly, this rejection may be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is requested. A marked-up version indicating the amendments made to the claims, as required by 37 C.F.R. § 1.121(c)(1)(ii), is enclosed. Also enclosed is a petition to extend the period for replying for four months, to and including February 13, 2002, a check in the amount of $720.00 for the fee required.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: January 28, 2002

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Anthony Giordano et al.	Art Unit:	1636
Serial No.:	09/437,458	Examiner:	G. Leffers, Jr.
Filed:	November 10, 1999	Customer No.:	21559

C.P.A. filed: January 28, 2002

Title: NUCLEIC ACID SEQUENCES AND METHODS FOR IDENTIFYING COMPOUNDS THAT AFFECT RNA/RNA BINDING PROTEIN INTERACTIONS AND mRNA FUNCTIONALITY

Box CPA
Assistant Commissioner for Patents
Washington, D.C. 20231

Version with Markings to Show Changes Made

Marked-up versions of claims 3 and 12-31 and new claims 32-34 are presented below.

3. (Amended) The isolated fusion nucleic acid sequence of claim 32 [1], wherein the regulation of mRNA functionality comprises an alteration in pre-mRNA processing or in the stabilization, translational efficiency, localization, sequestration, editing, or splicing functions of said mRNA form of said fusion nucleic acid.

12. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 1 [or a subfragment nucleic acid derived from SEQ ID NO: 1].

13. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide

sequence of said first nucleic acid comprises SEQ ID NO: 2 [or a subfragment nucleic acid derived from SEQ ID NO: 2].

14. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 3 [or a subfragment nucleic acid derived from SEQ ID NO: 3].

15. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 4 [or a subfragment nucleic acid derived from SEQ ID NO: 4].

16. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 5 [or a subfragment nucleic acid derived from SEQ ID NO: 5].

17. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 6 [or a subfragment nucleic acid derived from SEQ ID NO: 6].

18. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 7 [or a subfragment nucleic acid derived from SEQ ID NO: 7].

19. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 8 [or a subfragment nucleic acid derived from SEQ ID NO: 8].

20. he isolated nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 9 [or a subfragment nucleic acid derived from SEQ ID NO: 9].

21. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 10 [or a subfragment nucleic acid derived from SEQ ID NO: 10].

22. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 11 [or a subfragment nucleic acid derived from SEQ ID NO: 11].

23. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 12 [or a subfragment nucleic acid derived from SEQ ID NO: 12].

24. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 13 [or a subfragment nucleic acid derived from SEQ ID NO: 13].

25. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 14 [or a subfragment nucleic acid derived from SEQ ID NO: 14].

26. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 15 [or a subfragment nucleic acid derived from SEQ ID NO: 15].

27. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 16 [or a subfragment nucleic acid derived from SEQ ID NO: 16].

28. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 17 [or a subfragment nucleic acid derived from SEQ ID NO: 17].

29. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 18 [or a subfragment nucleic acid derived from SEQ ID NO: 18].

30. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 19 [or a subfragment nucleic acid derived from SEQ ID NO: 19].

31. (Amended) The isolated fusion nucleic acid of 32 [1], wherein the nucleotide sequence of said first nucleic acid comprises SEQ ID NO: 20 [or a subfragment nucleic acid derived from SEQ ID NO: 20].

32. (New) An isolated fusion nucleic acid, comprising a first nucleic acid operably linked to a heterologous second nucleic acid, wherein said first nucleic acid comprises any one of SEQ ID NOS: 1-20, and wherein the mRNA form of said first nucleic acid has RNA binding protein (RBP) binding activity or regulates the functionality of the mRNA form of said fusion nucleic acid.

33. (New) The isolated fusion nucleic acid of claim 32, wherein said fusion nucleic acid is DNA or cDNA.

34. (New) The isolated fusion nucleic acid of claim 32, wherein said fusion nucleic acid is mRNA.

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